```
RESULT   3
AAR37424
ID   AAR37424 standard; Protein; 490 AA.
XX
AC   AAR37424;
XX
DT   28-SEP-1993  (first entry)
XX
DE   Human CTR.
XX
KW   Human; calcitonin receptor; CTR; cell membrane; small cell; probe;
KW   ovarian; carcinoma; cell line; BIN-67; cAMP; porcine; E. coli.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1..22
FT                   /note= "Signal peptide"
FT   Protein         23..490
FT                   /note= "Mature protein"
FT   Modified-site   28
FT                   /note= "N-linked glycosylation site"
FT   Misc-difference 55
FT                   /note= "Extracellular Cys"
FT   Misc-difference 72
FT                   /note= "Extracellular Cys"
FT   Modified-site   73
FT                   /note= "N-linked glycosylation site"
FT   Misc-difference 81
FT                   /note= "Extracellular Cys"
FT   Misc-difference 95
FT                   /note= "Extracellular Cys"
FT   Misc-difference 112
FT                   /note= "Extracellular Cys"
FT   Modified-site   125
FT                   /note= "N-linked glycosylation site"
FT   Modified-site   130
FT                   /note= "N-linked glycosylation site"
FT   Misc-difference 134
FT                   /note= "Extracellular Cys"
FT   Domain          147..175
FT                   /note= "Transmembrane domain I"
FT   Domain          203..225
FT                   /note= "Transmembrane domain II"
FT   Misc-difference 235
FT                   /note= "Extracellular Cys"
FT   Domain          253..272
FT                   /note= "Transmembrane domain III"
FT   Domain          280..301
FT                   /note= "Transmembrane domain IV"
FT   Misc-difference 305
FT                   /note= "Extracellular Cys"
FT   Domain          313..340
FT                   /note= "Transmembrane domain V"
FT   Domain          360..377
FT                   /note= "Transmembrane domain VI"
FT   Domain          394..411
FT                   /note= "Transmembrane domain VII"
XX
PN   WO9310149-A.
XX
PD   27-MAY-1993.
XX
PF   09-NOV-1992;   92WO-US09686.
XX
PR   15-NOV-1991;   91US-0792885.
XX
PA   (GEHO ) GEN HOSPITAL CORP.
XX

CC   The present peptide, which promotes osteoblast proliferation and
CC   enhances osteoblast activity, can be used to treat bone diseases
CC   when administered at a dose of 0.1-10 mg/day.
CC   The percentage increase in cell numbers in rat osteoblast ROS
CC   cell cultures following treatment with 1, 0.1 or 0.01 micorg of
CC   peptide/well was 1317.1, 636.2 and 110.5, compared to 100 for an
CC   untreated control.
XX
SQ   Sequence   16 AA;

  Query Match           100.0%;  Score 87;  DB 17;  Length 16;
  Best Local Similarity  100.0%;  Pred. No. 3.5e-08;
  Matches   16;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy       1 KLTTIFPLNWKYRKAL 16
           ||||||||||||||||
Db       1 klttifplnwkyrkal 16
```

> seq id No 1

Seq. comp. C

CC This is a calcitonin receptor peptide fragment that can be used in the
CC method of invention of searching for physiologically active substances.
CC The method comprises analysing the amino acid sequence of receptors
CC having at least 2 members with different sizes of the same type, and
CC examining which regions in the longer receptor is missing in the shorter
CC one. The receptors are for a substance where an antagonist occurs in
CC vivo. The method is useful for the preparation of physiologically active
CC peptides. The method, which doesn't require isolation of physiologically
CC active substances permits highly efficient searching of physiologically
CC active substances.
XX
SQ Sequence 12 AA;

Query Match 100.0%; Score 74; DB 19; Length 12;
Best Local Similarity 100.0%; Pred. No. 3.3e-05;
Matches 12; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 PSCQWVQAPACQ 12
||||||||||||
Db 1 pscqwvqapacq 12

RESULT 2
AAY50564
ID AAY50564 standard; peptide; 12 AA.
XX
AC AAY50564;
XX
DT 25-JAN-2000 (first entry)
XX
DE Growth hormone secterion promoting peptide.
XX
KW Insulin; inhibitor; treatment; diabetes; regulator; secretion;
KW growth hormone production; modulator; gastric acid.
XX
OS Synthetic.
XX
PN WO9951627-A1.
XX
PD 14-OCT-1999.
XX
PF 05-APR-1999; 99WO-JP01796.
XX
PR 04-APR-1998; 98JP-0108662.
PR 08-APR-1998; 98JP-0112819.
XX
PA (NAKO/) NAKOSHI H.
PA (SAKA/) SAKAMOTO K.
XX
PI Sakamoto K;
XX
DR WPI; 1999-633728/54.
XX
PT Effective method for searching physiologically-active substances under
PT certain predictability, e.g. peptide drugs in remedies for diabetes and
PT diseases of insulin-production regulation and gastric secretion -
XX
PS Claim 15; Page 19; 23pp; Japanese.
XX
CC This invention describes a novel method for searching physiologically
CC active substances. The screened substances can be used to treat
CC diabetes, regulate insulin production, inhibit gastric secretion and
CC modulate growth hormone production. AAY50559-Y50564 represent peptides
CC used to inhibit or regulate insulin-production, gastric acid secretion
CC and growth hormone secretion and are used in the method of the
CC invention.
XX
SQ Sequence 12 AA;

Query Match 100.0%; Score 74; DB 20; Length 12;
Best Local Similarity 100.0%; Pred. No. 3.3e-05;
Matches 12; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 PSCQWVQAPACQ 12
||||||||||||
Db 1 pscqwvqapacq 12

RESULT 3
AAR39264
ID AAR39264 standard; Protein; 428 AA.
XX
AC AAR39264;
XX
DT 18-NOV-1993 (first entry)
XX
DE Murine somatostatin receptor-3.
XX
KW Mouse; somatostatin; receptor; SSTR-1; SSTR-2; SSTR-3; tumour;
KW pancreas; islet; promoter; transformation; host cell.
XX
OS Mus musculus.
XX
PN WO9313130-A.
XX
PD 08-JUL-1993.
XX
PF 30-DEC-1992; 92WO-US11327.
XX
PR 31-DEC-1991; 91US-0816283.
XX
PA (ARCH-) ARCH DEV CORP.
XX
PI Bell GI, Seino S, Yamada Y;
XX
DR WPI; 1993-227272/28.
DR N-PSDB; AAQ45658.
XX
PT Somatostatin receptors useful for diagnosis of tumours - also
PT useful for screening candidate somatostatin receptor agonists and
PT antagonists
XX
PS Claim 3; Page 76-77; 94pp; English.
XX
CC The sequences given in AAR39260, AAR39262 and AAR39264 represent the
CC murine somatostatin receptors (SSTR)-1, SSTR-2 and SSTR-3. The DNA
CC encoding these proteins was isolated from total murine pancreatic islet
CC DNA. These DNA sequences may be placed under the control of a suitable
CC promoter and used to transform a host cell. The DNA sequences and
CC these proteins may be used in screening assays for testing candidates
CC including agonists and antagonists of SSTR polypeptides. The assays
CC may be used to discriminate candidate substances with desirable
CC properties specific to SSTR polypeptides. The isolated substances
CC may be used in a wide range of aplications eg. diagnosis of various
CC human tumours. Fragments of these DNA sequences may be used as
CC probes in the isolation of other SSTR-encoding clones.
XX
SQ Sequence 428 AA;

Query Match 100.0%; Score 74; DB 14; Length 428;
Best Local Similarity 100.0%; Pred. No. 0.001;
Matches 12; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 PSCQWVQAPACQ 12
||||||||||||
Db 244 pscqwvqapacq 255

Seq. comp. D
Seq ia. No. 3

RESULT 4
AAY79282
ID AAY79282 standard; Peptide; 8 AA.
XX
AC AAY79282;

```
                                                                    <------ M1 -----
mSSTR1  1 MFPNGTASSPSSSPSPSPGSCGEGACSRGPGSGAADGMEEPGRNASQNGTLSEGQGSAILISFIYSVVCLVG
mSSTR2A                 1 MEMSSEQLNGSQVWVSSPFDLNGSLGPSNGSNQTEPYYDMTSNAVLTFIYFVVCVVG
mSSTR2B                 1 MEMSSEQLNGSQVWVSSPFDLNGSLGPSNGSNQTEPYYDMTSNAVLTFIYFVVCVVG
mSSTR3                1 MATVTYPSSEPMTLDPGNTSSTWPLDTTLGNTSAGASLTGLAVSGILISLVYLVVCVVG
rSSTR4                      1 MEPLSLASTPSWNASAASSGNHNWSLVGSASPMGARAVLVPVLYLLVCTVG
rSSTR5                    1 MNTPATLPLGGEDTTWTPGINASWAPDEEEDAVRSDGTGTAGMVTIQCIYALVCLVG

          ----------->          <--------- M2 -------->                    <-- M3 ----
mSSTR1    LCGNSMVIYVILRYAKMKTATNIYILNLAIADELLMLSVPFLVTSTLLRH:WPFGALLCRLVLSVDAVNMFT
mSSTR2A   LCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLAMQVALVH:WPFGKAICRVVMTVDGINQFT
mSSTR2B   LCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLAMQVALVH:WPFGKAICRVVMTVDGINQFT
mSSTR3    LLGNSLVIYVVLRHTSSPSVTSVYILNLALADELFMLGLPFLAAQNALSY:WPFGSLMCRLVMAVDGINQFT
rSSTR4    LSGNTLVIYVVLRHAKMKTVTNVYILNLAVADVLFMLGLPFLATQNAVVSYWPFGSFLCRLVMTLDGINQFT
rSSTR5    LVGNALVIFVILRYAKMKTATNIYLLNLAVADELFMLSVPFVASAAALRH:WPFGAVLCRAVLSVDGLNMFT

          --------->              <--------- M4 --------->
mSSTR1    SIYCLTVLSVDRYVAVVHPIKAARYRRPTVAKVVNLGVWVLSLLVILPIVVFSRTAANSDGT:VACNMLMPE
mSSTR2A   SIFCLTVMSIDRYLAVVHPIKSAKWRRPRTAKMINVAVWCVSLLVILPIMIYAGLRSNQWGR:SSCTINWPG
mSSTR2B   SIFCLTVMSIDRYLAVVHPIKSAKWRRPRTAKMINVAVWCVSLLVILPIMIYAGLRSNQWGR:SSCTINWPG
mSSTR3    SIFCLTVMSVDRYLAVVHPTRSARWRTAPVARTVSRAVWVASAVVVLPVVVFSGVP:::RGM:STCHMQWPE
rSSTR4    SIFCLMVMSVDRYLAVVHPLRSARWRRPRVAKMASAAVWVFSLLMSLPLLVFADVQ:EGWGT:::CNLSWPE
rSSTR5    SVFCLTVLSVDRYVAVVHPLRAATYRRPSVAKLINLGVWLASLLVTLPIAVFADTRPARGGEAVACNLHWPH

          <---------- M5 ---------->
mSSTR1    PAQRWLVGFVLYTFLMGFLLPVGAICLCYVLIIAKMRMVALKAGW:::::::::::QQRKRSERKITLMVMM
mSSTR2A   ESGAWYTGFIIYAFILGFLVPLTIICLCYLFIIIKVKSSGIRVGS:::::::::::SKRKKSEKKVTRMVSI
mSSTR2B   ESGAWYTGFIIYAFILGFLVPLTIICLCYLFIIIKVKSSGIRVGS:::::::::::SKRKKSEKKVTRMVSI
mSSTR3    PAAAWRTAFIIYMAALGFFGPLLVICLCYLLIVVKVRSTTRRVRAPSCQWVQAPACQRRRRSERRVTRMVVA
rSSTR4    PVGLWGAAFITYTSVLGFFGPLLVICLCYLLIVVKVKAAGMRVGS:::::::::::SRRRRSEPKVTRMVVV
rSSTR5    P::AWSAVFVIYTFLLGFLLPVLAIGLCYLLIVGKMRAVALRAGW:::::::::::QQRRRSEKKITRLVLM

          -------- M6 -------->          <--------- M7 ---------->
mSSTR1    VVMVFVICWMPFYVVQLVNVF::AEQDDATVSQ::LSVILGYANSCANPILYGFLSDNFKRSFQRILCLSWM
mSSTR2A   VVAVFIFCWLPFYIFNVSSVSVAISPTPALKGMFDFVVILTYANSCANPILYAFLSDNFKKSFQNVLCLVKV
mSSTR2B   VVAVFIFCWLPFYIFNVSSVSVAISPTPALKGMFDFVVILTYANSCANPILYAFLSDNFKKSFQNVLCLVKA
mSSTR3    VVALFVLCWMPFYLLNIVNVVCPLPEEPAFFGLYFLVVALPYANSCANPILYGFLSYRFKQGFRRILLRPSR
rSSTR4    VVLVFVGCWLPFFIVNIVNLAFTLPEEPTSAGLYFFVVVLSYANSCANPLLYGFLSDNFRQSFRKVLCLRRG
rSSTR5    VVTVFVLCWMPFYVVQLLNLFV::TSLDATVNH::VSLILSYANSCANPILYGFLSDNFRRSFQRVLCLRCC

mSSTR1    DNAAEEPVDYYATALKSRAYSVEDFQPENLESGGVFRNGTCASRISTL - 391
mSSTR2A   SGTEDGERSDSKQDKSRLNETTETQRTLLNGDLQTSI - 369
mSSTR2B   DNSQSGAEDIIAWV - 346
mSSTR3    RIRSQEPGSGPPEKTEEEEDEEEEERREEEERRMQRGQEMNGRLSQIAQAGTSGQQPRPCTGTAKEQQLLPQ
rSSTR4    YGMEDADAIEPRPDKSGRPQATLPTRSCEANGLMQTSRI - 363
rSSTR5    LLETTGGAEEEPLDYYATALKSRGGPGCICPPLPCQQEPMQAEPACKRVPFTKTTTF - 384

mSSTR3    EATAGDKASTLSHL - 428
```

Fig. 1. Comparison of amino acid sequences of the cloned SRIF receptors. The sequences of the cloned mouse (*m*) and rat (*r*) subtypes are shown. Invariant residues are shown in *boldface* type. *Colons*, gaps introduced to generate this alignment. The seven predicted transmembrane domains (*M1-M7*) are shown. The sequences are from Refs. 4–8.

TABLE 1

Affinity of SSTR2A and SSTR2B for SRIF analogs

Values are the means of three different experiments, and the standard error was <10% of the mean.

Compound	IC_{50}	
	SSTR2A	SSTR2B
	nM	
D-Trp[8]-SRIF	0.001	0.001
MK-678	0.01	0.01
SMS-201-995	0.4	0.2
BIM 23023	0.001	0.001
BIM 23027	0.001	0.001
BIM 23034	0.001	0.001
NC4-28B	0.001	0.001
L362-862	0.23	0.6
L363-572	6.0	8.5

In contrast, SRIF did not inhibit cAMP formation in cells expressing SSTR2A. Because SSTR2A and SSTR2B differ in sequence in only a limited region at their carboxyl termini, this finding implicates this region of SSTR2 in coupling to adenylyl cyclase.

Experimental Procedures

Materials. SRIF and SRIF-28 were obtained from Bachem (Torrance, CA). MK-678, L-363,572, and L-362,862 were the gifts of Dr. D. Veber (Merck, West Point, PA). SMS-201-995 was obtained from Sandoz (Basel, Switzerland). All other peptides were the gifts of Dr. D. Coy (Tulane University, New Orleans, LA) and Biomeasure, Inc. (Hopkinton, MA).

Cloning of mouse SSTR2B. A SSTR2B cDNA construct was engineered by the PCR-based strategy, using SSTR2A cDNA as a template. The 3′ half of SSTR2A cDNA was first PCR amplified with oligo-$m_2$51 (nucleotides 1191–1210 of SSTR2B) and oligo-$m_2$52 (nucleotides 1557–1579 of SSTR2B). To generate a corresponding fragment for the 3′ half of SSTR2B cDNA, the PCR product was reamplified with oligo-$m_2$51 and oligo-$m_2$50 (nucleotides 1557–1629 of SSTR2B), tagged with a *Bam*HI site at the 5′ end of the primer; oligo-$m_2$50 covers the divergent region between SSTR2A and SSTR2B. The PCR was carried out for 25 cycles of denaturation at 95° for 1 min, annealing at 55° for 1 min, and extension at 72° for 1 min, using GeneAmp reagents. The amplified fragments were digested with *Kpn*I and *Bam*HI and subcloned into pGEM3Z (yielding pGEM3Z-3′2B). The *Xba*I/*Kpn*I fragment from SSTR2A and the *Kpn*I/*Sal*I fragment from pGEM3Z-3′2B were subcloned into the *Xba*I/*Sal*I site of pCMV6C to generate pCMV-SSTR2B. The sequence of this fragment was identical to the published SSTR2B cDNA sequence (8). Both cDNAs were transfected into COS-7 cells as described previously (5, 16).

Receptor binding assay. Binding studies were performed using the same procedures as described previously (14, 16). Cells were harvested 72 hr after transfection in 50 mM Tris·HCl, pH 7.8, containing 1 mM EGTA, 5 mM $MgCl_2$, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 200 μg/ml bacitracin, and 0.5 μg/ml aprotinin (buffer 1) and were centrifuged at 24,000 × *g* for 7 min at 4°. The pellet was homogenized in buffer 1 using a Brinkman Polytron (setting 2.5, 30 sec). The homogenate was then centrifuged at 48,000 × *g* for 20 min at 4°. The

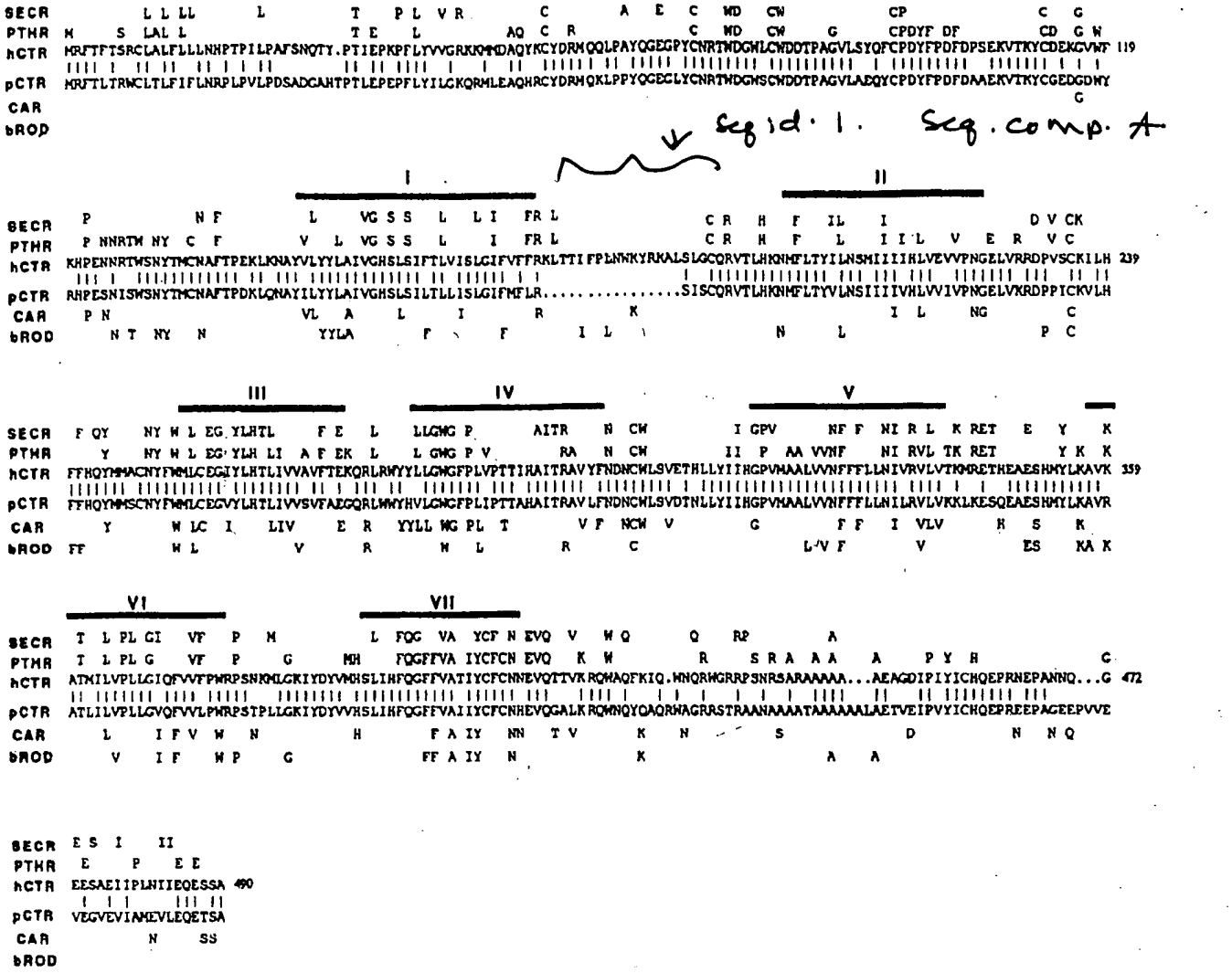

Figure 6. Alignment of the hCTR to the pCTR and to other G protein–coupled receptors. The complete hCTR and pCTR sequences are compared; gaps for this alignment are indicated by dots and identical residues indicated by vertical lines. Identities of the hCTR and the closely related opossum PTH–PTHrp (*PTHR*) and rat secretin receptor (*SECR*) are indicated above the CTR sequences. Identities of the hCTR with the *D. discoideum* CAR and bROD are indicated below the CTR sequences. The alignment of the CAR NH_2 terminus relative to the longer hCTR sequence begins at hCTR residue 115 and the alignment ends with CAR residue 362 aligned with the hCTR COOH terminus (hCTR residue 490). The bROD NH_2 terminus alignment begins with hCTR residue 123 and the alignment ends with bROD residue 349 aligned with hCTR residue 449. The solid lines above the sequences indicate the proposed transmembrane domains for the hCTR, labeled I–VII.

higher than the 12–20% identity found among the other principal families that comprise the superfamily of G protein–coupled receptors (42).

Each of the closely related peptide-binding receptors for CT, PTH–PTHrp and secretin possess homologous signal peptide-like NH_2-terminal domains. The six cysteines in the first extracellular domain of the hCTR and pCTR are conserved and require no gap insertions for their alignment. The positions of the five cysteines closest to the carboxy-terminal end are conserved in the PTH–PTHrp and CT receptors, but only four of these cysteines are conserved in the secretin receptor. In addition, two other extracellular cysteines are notably conserved at sites in the putative second and third extracellular domains of all three receptor types. Three of four potential *N*-linked glycosylation sites in the first extracellular domains are preserved in the hCTR and pCTR; the two sites closest to

Figure 5. Nucleotide and predicted amino acid sequence of the hCTR cDNA. The underlined nucleotide triplet indicates a potential initiation codon upstream of the assigned putative translation start site (see text). The arrow indicates a potential cleavage site (between amino acids 22 and 23) for a hydrophobic leader sequence. Four potential *N*-linked glycosylation sites are indicated by shaded circles. Open circles indicate cysteines in the first extracellular loop and the conserved cysteines in the second and third extracellular loops. The seven putative hydrophobic membrane-spanning domains are also underlined.